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ON THE LEAF AND SPOROCARP OF PILULARIA.

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(WITH PLATES I–III)

DURING the course of some work on the development of the foliar structures of *Marsilia quadrifolia* L., the results of which have been published elsewhere ('98), a brief examination was also made of the same structures in the related Pilularia. Certain features observed here suggested the desirability of making a more careful study of the latter genus, and following out in detail all stages of the development for comparison with the same stages in Marsilia.

Through the kindness of Professor Eugenius Warming, Dr. Ostenfeld Hansen, of Copenhagen, collected for me in July 1897 a considerable quantity of fruiting material of *Pilularia globulifera* L. With this material the work embodied in the present paper has been prosecuted at the biological laboratory of the Johns Hopkins University, while holding the Adam T. Bruce fellowship for 1897–8.

The material was fixed in 95 per cent. alcohol, which seemed to give very good results except in the older stages, where the preparation of certain tissues of the capsule for the gelatinization, which causes the bursting of the capsule, had already begun. In this stage the sporocarps were often considerably shrunken, as happens in Marsilia after using other fixing agents unless great care is used in running up through the alcohols.

Sections of the structures studied were cut in paraffine, stained rather lightly in Mayer's haemalum, and then strongly with Bismarck brown in saturated solution in 70 per cent. alcohol.

THE LEAF.

The leaves of Pilularia arise, in acropetal succession, on the right and left sides alternately of the upper surface of the stem. Each leaf originates in a large cell from which a typical two-sided apical cell is cut out by curved anticlines. The apical cell thus formed has its longer axis directed toward the stem apex (*L, fig. 1*), as was shown by Bower ('84).

This apical cell swells out beyond the general surface of the stem and cuts off segments, alternately toward the right and left of the latter and of the leaf itself. The number of segments formed is probably about fifteen pairs. In several young leaves where the segments could be counted the number was found to be ten or eleven on each side of the apical cell, but in older leaves where the segments could not be satisfactorily counted the number was apparently considerably greater, at least as many as fifteen.

By the growth and division of these segments a papilla-like organ is formed, which soon begins to curve in ventrally (*L, fig. 3*) toward and above the stem apex. This circinate coiling continues with growth of the leaf until, when a centimeter long, the tip may form a flat spiral of two turns or more, of which the inner projects out laterally beyond the plane of the outer, so that median sagittal sections cannot be obtained through the whole length of the leaf.

Up to quite a late period the apical cell can be distinguished, but whether its fate is finally like that to be described for the apical cell of the sporocarp was not determined. The shape of this is never three-sided, as described by Campbell ('93) in *P. Americana*, but is two-sided as stated by Meunier ('87), though the segments are not cut off toward the dorsal and ventral sides of the leaf as seems to be indicated in Meunier's figures, but to the right and left alternately as we have seen.

The primary division of the semicircular segments or "primary marginal cells" is quite regular and at first resembles exactly that described by Poirault (**'90**) and myself for Marsilia. The first wall formed is a longitudinal and radial anticline (*I, figs. 3, 4*) cutting off about one-third of the segment toward the dorsal side to form what we may call a section. The second is a similar wall (*II, figs. 2–4*) forming a second section next to the median wall of the leaf (or inner border of the segment), and leaving a "tertiary marginal cell" (*m* c^3, *fig. 4*). Then a transverse anticline (*t* a^1, *figs. 2, 3*) divides this marginal cell into two, an upper and a lower one. In each of these tertiary marginal cells a third longitudinal anticline appears (*III, figs. 3, 5*) nearly parallel to wall I. At this point the similarity to Marsilia ceases, for instead of forming two more sections, as in that plant, each marginal cell of the fourth grade is here divided by a pericline (*d w, fig. 6*), thus ending its function as a marginal cell.

The four primary divisions formed in each segment (leaving out of account the transverse anticlines which do not appear in a cross section of the leaf) develop in a way very like that found in the six primary divisions in the segments of the leaf of Marsilia. Section I very early cuts off by a pericline near the inner end (*p l w, fig. 4*) a cell which is to function as procambium. The outer end of the section is soon cut in halves by a longitudinal anticline (*h a, fig. 4*). Then sections II and III and the marginal cell form procambium at their inner ends (*p l w, fig. 6*), while at the outer ends of all the sections periclines, corresponding to that in the marginal cell, separate the protoderm and ground meristem layers which encircle the procambium (*figs. 6, 7*). No second portion of procambium is ever formed in section I, as happens in Marsilia.

The protoderm layer soon divides by periclines into epidermis and hypodermis (*e p* and *h y, figs. 7–9*) and the cells of these then divide by anticlines to form many cells, but each layer remains of a single cell in thickness even at maturity, as no more periclines are ever formed. The procambium throughout breaks up by numerous longitudinal walls and fewer transverse ones to

form the elongated cells of the axial vascular bundle (*a b, figs. 6–9*). Of the cells arising thus, one of the four first formed in the procambium of section II (*t r, figs. 6–9*) develops without further division by longitudinal walls into the large trachea of each side of the bundle. Whether transverse anticlines are formed in this in its later development could not be made out with certainty, but it agrees apparently with the trachea of the same bundle in Marsilia in remaining the full length of the segment. As the bundle progresses in its development the outer layer of the cells formed from the procambium becomes specialized as an endodermis or bundle sheath (*b s, figs. 8, 9*). The fact that the first wall formed in the procambium of section I does not correspond exactly with the halving anticline in the outer end of this section (*fig. 5*) makes it easy to distinguish procambium and ground meristem at this point (*figs. 6–8*). The leaf of Pilularia thus forms an exception to the general rule holding in the ferns, that the endodermis is formed from the ground meristem surrounding the bundle (Haberlandt, **'96,** p. 336); and agrees rather with the Juncaceæ and Cyperaceæ which have been studied by Haberlandt. I am inclined also to believe that Marsilia agrees with Pilularia in this respect, though it was not possible to determine this with absolute certainty as in the present case.

We may now turn to follow briefly the development of the ground meristem layer. The two primary cells of this layer in section I and the single cell of each of the other three main divisions of the segment divide by a single pericline in each. The inner layer of cells thus formed, ten in number, constitutes the mesophyll layer (*m p, figs. 7–9*), which remains of this number (as seen in cross section) until maturity, being one cell thick and having no tannin sacs like those of Marsilia. Of the ten outer cells (*p c, figs. 7–9*), of which there are at this time several in the length of a segment, each gives rise to its part of one of the ten longitudinal partitions which separate the ten longitudinal air canals (*a c, figs. 7, 9*), in the leaf of Pilularia (Bischoff, **'28**). These air canals arise very early as small

intercellular spaces between the cells of the ground meristem and those of the hypodermis (*a c*, *figs. 7–9*) on the line of the median wall, of each section wall, and on the halving anticline of section I. The primary partition cells divide by periclines and thus increase in a radial direction as the leaf increases in diameter. At certain points the tips of these cells remain in contact with those of their fellows in adjoining sections laterally (as at the median wall and halving anticline in *fig. 8*). When viewed in tangential section (*pc*, *fig. 10*) it is seen that each cell of the partition elongates tangentially and forms thus a protuberance at the upper end on one side and at the lower end of the cell on the other side. Then when the next transverse anticline is formed it is slightly oblique and forms two wedge-like cells, each with a protuberance on one end and none at the other (*fig. 10*). These cells soon elongate with the growth in length of the leaf, and cells are cut off from each which are not in contact with their fellows laterally (*fig. 11*). Thus arise the longitudinal partitions which separate laterally the adjacent air canals. The protruding ends of the partition cells, which separate the divisions, at first short, of the same longitudinal canal from each other, are finally cut off by oblique anticlines (*c p*, *fig 11*) and form thus transverse partitions two cells broad from one longitudinal partition to the next. These cells do not divide further, as in Marsilia to form transverse partitions many cells in width, but elongate very greatly as the longitudinal partitions separate by the growth in circumference of the leaf. The latter, however, are closer together at the point where joined by the transverse partitions, and thus each longitudinal partition, as seen in tangential section, has a zigzag course from the base to the apex of the leaf. Both kinds of partitions remain one cell in thickness throughout, and both are perforated by pores, the "meats" of Meunier, which allow the free circulation of the enclosed air to all parts of the leaf.

The stomata which are present on both the leaf and the sporocarp and the peculiar trichomes which cover all the younger parts (Mettenius '46) have been carefully studied and figured

by Meunier, and for the sake of simplicity have been omitted from nearly all drawings in the present paper. We may note, however, that the origin of the trichome from a portion cut from the acroscopic end of an epidermal cell is practically the same as in Marsilia, though the regularity is not so striking.

As is well known (Bischoff '28), the mature leaf of Pilularia has no lamina whatever. We may consider it as quite probable, however, that the immediate ancestors of Pilularia and Marsilia had leaves possessing a lamina, which still persists in the latter genus, and we might expect to find some remnant or trace of this in Pilularia in the mode of division of the segments if not in the outward form. Keeping in mind then the mode of development of the lamina in Marsilia, by the continued activity of the marginal cells near the apex of the leaf, many segments in this region of the leaf of Pilularia were examined in search of any irregularity in the formation of cell walls, or of continued activity of the marginal cells. The results were always negative, and we must therefore conclude that the development of the leaf of Pilularia gives no indication that it has ever possessed a lamina.

THE SPOROCARP.

The sporocarp of Pilularia was considered by Hofmeister ('62) as a modified branch arising as an accessory bud at the base of the leaf. Alexander Braun ('70) and his pupil Russow ('72) dissent from this view, and on theoretical grounds consider it a segment or branch of the leaf; while Juranyi ('80) and Goebel ('82) confirm this latter view as a result of the study of the development of the sporocarp and the relation of its tissues to those of the neighboring leaf. Meunier ('87), however, while holding that the latter view is probably the correct one, thinks the evidence adduced is at fault, as the vascular bundle of the sporocarp, according to his observations, does not fuse first with that of the leaf, but with that of the stem itself. He then points out that the best evidence for this view will be the proof that both arise from the single apical cell of a foliar structure at first

unbranched. This he was not fortunate enough to obtain, and it was left for Campbell ('93) to show that this was the case in *P. Americana*, where he found the sporocarp arose from a single cell at the base of the leaf. In the development of the capsule of the sporocarp in Pilularia, Juranyi thought the soral cavities arose by a splitting of the internal tissues of the young capsule, as Russow had described for Marsilia. Goebel on the contrary held that these cavities were external in origin, and this view was later confirmed by the work of Meunier and Campbell.

According to Meunier's work the young sporocarp is developed from a two-sided (possibly a three-sided) apical cell which soon ceases to function as such, and growth is continued by the activity of four cells occupying the four corners of the tip of the sporocarp. Each of these cells was supposed to give rise to one of the four valves of the mature capsule with its sorus.

Meunier's figures of the vascular bundle system of the capsule and of the stalk show that the sporocarp is bilaterally symmetrical, and that the plane of symmetry separates the sori into a right pair and a left pair and does not pass through the middle of diagonally opposite sori as do the longitudinal sections figured by Meunier.

The latest work on the development of the capsule (Campbell '93 and '95) indicates that one of the valves or lobes of the young capsule is developed directly from the apical cell of the sporocarp, being thus terminal in position, while a second appears lower down on the median line of the side toward the leaf, and the third and fourth on the right and left of this line respectively. This of course means that the plane of symmetry must pass through the upper and lower sori and between the other two, which does not agree well with the structure of the mature capsule of Pilularia as given by Meunier, or with the mode of development that I found in Marsilia. It was this difficulty in seeing how the structure of the mature capsule as given by Meunier could be developed in the manner described by Campbell that led me to take up the present work.

According to my own observations the sporocarp of Pilularia

arises on the inner and anterior side of the leaf, just above the axillary bud which is always present. As in Marsilia, a fertile branch of the stem has a sporocarp on nearly every leaf, but there is never more than one on the same leaf in Pilularia, or could any rudiment of a second be found.

The young sporocarp owes its origin to the formation of a two-sided apical cell in one of the marginal cells of the fourth grade in (probably) the first segment of the anterior side of the leaf (*F*, *fig.* 12). The difficulties of orientation were such that transverse sections of the leaf in this region were not frequently obtained, and it cannot be definitely stated therefore that the sporocarp arises in the quaternary marginal cell, rather than the tertiary one, but the evidence obtained seems in favor of the former. The fusion of the outer tissues of the leaf with the stem makes it impossible also to state positively from which segment the sporocarp arises, but I believe it to be the first rather than the second, and certainly it cannot be a younger one than this.

The apical cell of the sporocarp has its longer axis across the leaf, and cuts off segments toward the base and apex of the leaf alternately, to the number of seven or more on each side (*figs.* 13, 14). The exact number of segments formed could not be determined with certainty, but in several cases six and seven pairs were counted, and in others very little older, as shown by their size, apical growth had ceased, and the number of segments was certainly much smaller than in Marsilia, where I found more than twenty pairs of segments. The activity of the apical cell as such is ended when the sporocarp is about a tenth of a millimeter long, by the appearance in it of several irregular anticlines, dividing it up into small cells which soon become indistinguishable from those derived from its later segments, as can be readily seen in a surface view of this part of the capsule (*fig.* 15).

The fate of the segments of the apical cell in the sporocarp is at first exactly like that of the leaf segments (*figs.* 13, 14, 16, 17), but while in the leaf wall III is followed by a pericline that cuts off the protoderm in the marginal cell, it is here followed

by three more section walls. Wall IV is dorsal to the marginal cell and nearly parallel to wall III (*fig. 18*), wall V is on the ventral, and VI on the dorsal side of the marginal cell (*figs. 18, 19*), and the ultimate marginal cell is thus of the seventh grade, just as in the capsule of Marsilia (Johnson '98).

THE STALK.

The type of primary division just given is the one found in most of the later segments of the sporocarp, but in those at the base, which form the stalk, wall IV is often followed immediately by a pericline in the marginal cell which ends its activity as such (*fig. 20*). The further fate of the various sections and the marginal cell is quite similar to that found in the leaf. Procambium, ground meristem and protoderm layers are formed in all; the latter gives rise to epidermis and hypodermis (*e p, h y, figs. 20, 21*), and the ground meristem to the two or three-layered mesophyll and to the partitions separating the small and irregular air canals. We find a notable difference in the fate of the procambium, for the eccentric vascular bundle of the stalk is developed entirely, or nearly so, from the procambium of section I (*a b, fig. 20*), while most of the procambium of the other divisions is devoted to the formation of the large stereome bundle which lies ventral to and partially surrounding the vascular bundle (*s c l, figs. 21, 31–33*). This fuses below with the central stereome of the stem, but ends abruptly above at the basal wall of the capsule. In the mature sporocarp the stalk is sharply curved in ventrally, is smaller at the lower end and considerably enlarged at the upper end where it joins the capsule, at which point also it is peculiarly modified on the dorsal side, as will be described in detail in speaking of the wall of the capsule.

The vascular bundle of the stalk, as Meunier has pointed out, does not fuse with that of the leaf in the way described by Goebel, but usually, according to my own observations, fuses first with the bundle of the axillary bud, and then this composite bundle reaches that of the stem at or near where the leaf bundle joins the latter.

THE CAPSULE.

In the terminal segments of the sporocarp, which form the capsule, the number of primary divisions is seven, as we have seen. Of these the six sections immediately divide up to form the three meristem layers, while the marginal cells, or at least a pair of these on each side, do not (*figs. 19, 22*). There are four ultimate marginal cells in each segment arising from the division of each of the two quaternary marginal cells by a transverse anticline (*fig. 14*). On each side of the capsule we find that two of these cells, in different but successive segments, become considerably larger than their fellows (*fig. 14*), and each finally gives rise to the sporangia of one of the four sori. In several cases these sporangial marginal cells, as we may call them, seemed to be the upper ones of the segments, as in the case figured (*fig. 14*), but the material at hand of this stage was not sufficient to allow me to determine whether this is always true. Neither can I assert positively that all of the sporangia of a sorus come from one marginal cell, but the evidence obtained is such that I feel practically satisfied that further study will show this to be the case. The essential thing, however, and one which is quite certain, is that the sporangia come from marginal cells, in a way that we shall find to be similar to that found in Marsilia, though differing in some details.

In describing further the development of the various structures of the capsule, we shall find it best to take them up separately, and may conveniently begin with the wall developed from the protodermal layer. Soon after apical growth ceases in the young sporocarp, the portion near the tip, that is the region including the four sporangial marginal cells, begins to swell out ventrally and laterally to form the globular capsule (*figs. 25, 31*), and these marginal cells at the same time begin to divide up to form the many sporangial cells of the sori. No tendency to a circinate coiling of the young sporocarp is seen at any time, but growth soon becomes more rapid on the lower side of the ventral protuberance and the original apex is thus pushed far

around dorsally (*A*, *figs. 31–33*), and the sori, which originate in cells having a lateral position (*fig. 14*), come later to have a position such that the soral canals open nearly terminally *figs. 31–33*), thus making the longitudinal axis of these canals nearly parallel to that of the stalk.

On the dorsal side of the sporocarp in the meantime there is formed a small protuberance at the upper end of the stalk (*l t*, *figs. 31, 32*), which is later found to be supported by a mass of thick-walled cells extending inward nearly to the vascular bundle (*fig. 33*). This protuberance, as was pointed out by Russow in *P. minuta*, is apparently homologous with the lower tooth of the capsule of *Marsilia quadrifolia*. Just above this tooth there is a rather narrow, but deep depression (*b p*, *figs. 31, 34*), which according to Russow corresponds to that found between the upper and lower teeth in *M. quadrifolia*, but there is no marked increase in height of the epidermal cells above this that might represent the upper tooth of the Marsilia capsule (see Russow '72, and Johnson '98).

It was this bending backward of the young capsule, perhaps, which led Meunier to think that the sori were primarily terminal in position, and this may account also for the view of Campbell that one of the valves is developed from the apical cell of the sporocarp, but it is not very difficult to follow out the details of development satisfactorily if the unchanging sagittal plane is used as a guide.

During this change in the general form of the capsule the protodermal layer throughout, beginning on the dorsal side just above the basal pit, divides by periclines into epidermal and hypodermal layers, and then the latter divides again to form inner and outer hypodermis (*e p*, *h y*, *figs. 30, 31*). These layers soon surround the whole capsule (Mettenius '46, Hanstein '66) except for the stomata, most of which are near the base of the capsule. At these openings both hypodermal layers are wanting, the guard cells being as usual derived from the epidermis (*s t*, *fig. 33*).

The development of these highly specialized tissue layers

has been very carefully studied by Meunier, and I will therefore give only a brief account of their mature structure. The brown walled cells of the epidermis are prismatic in shape, varying in height from one-half to three or four times their diameter, the highest being those at the base of the capsule near the ventral side (*fig. 33*). In the basal pit the epidermis is made up of several layers of irregular thin-walled cells (*figs. 32, 33*), while just above this on the wall of the capsule it consists of a single layer of very short cells (*fig. 33*). On either side of this narrow pit the epidermal cells are quite high, as was shown by Meunier, but, though this author figures transverse sections of this region of the capsule, he does not appear to have discovered the pit in longitudinal sections, and hence apparently failed to appreciate its significance. Scattered about among the epidermal cells of the ripe capsule are many of the persistent basal cells of the deciduous trichomes (*t c, fig. 33*).

The outer hypodermal layer consists of cells with the clear yellowish walls so thickened that the cavity is entirely obliterated except at the ends (*h y*^{1}, *figs. 33–35*). These cells are of quite uniform length and have the thick walls peculiarly modified at about the middle of their length, by the deposit, as Meunier thinks, of a more albuminous substance in this portion of the wall, to form the most prominent of the several "light lines" characteristic of this layer (*l l, figs. 33, 34*). The inner hypodermal layer consists of rather longer prismatic cells with thick walls, and are occasionally divided into inner and outer cells by obliquely transverse walls (*h y*^{2}, *fig. 33*). In the region between the stalk and capsule it is noticed, quite early in the development, that the cells are somewhat smaller than the surrounding ones (*b w, figs. 25, 31*), and the larger number of nuclei makes this quite striking in stained sections of a stage like that from which the latter figure is drawn, even when the power used is so low that the cell walls are not distinguishable. Finally, quite late in the development, one layer of these cells in the lower part of this basal wall become modified in the same manner as the outer hypodermis of the capsule (*fig. 33*), and

is continuous with this hypodermis laterally and ventrally (*figs. 33, 34*). For a narrow space in the dorsal region, however, the outer hypodermis from the dorsal wall of the capsule is seen in sagittal section to continue on down into the stalk, making a sharp bend and becoming much thickened just opposite the basal pit, while the similar layer of the basal wall laps over on to this above and abuts against the thin inner hypodermis (*fig. 33*). Horizontal sections (*fig. 35*) show that the region of overlapping is a very narrow one, and that this arises from the transverse division here of cells which are elsewhere undivided. Whether this division gives rise to an open slit is difficult to determine, but I believe that at maturity there is an actual opening here which may have the function, attributed by Russow to the similar structure in Marsilia, of allowing an interchange of air between the capsule and the air canals of the stalk.

Russow noticed the thinness and the bulging outward of the outer hypodermis just above the basal pit, as shown in horizontal section (*fig. 34*), but was unable to study it thoroughly from lack of material. The course of the light line at the point of overlapping is worthy of special notice. It moves toward the inner surface of the hypodermis (*ll*, *figs. 33, 34*) and finally passes over into the basal wall (*figs. 33, 35*), and the thickening of the walls of the cells in these layers is seen to be definitely related to this line, the cell cavity increasing in size with the distance out from this line toward the end of the cell (*figs. 34, 35*), while where two layers are present (*fig. 35*), the cells of the outer one, in which the light line is wanting, have very slightly thickened walls. At the stomata also the light line is seen to bend outward to the guard cells, so that we may conclude that this line indicates the distribution of some material which makes the hypodermis impervious to air or moisture, and is therefore present only where needed for this purpose.

At the base of the capsule the inner hypodermal layer is wanting, and is replaced by several layers of brown-walled cells which form the inner portion of the basal wall (*fig. 33*). Next to the prismatic outer layer of this wall these cells are closely

packed together, but farther up many small intercellular spaces occur. These spaces open into the larger spaces between the rounded parenchyma cells surrounding the vascular bundle under the base of the sori, and these in turn connect with the still larger canals in the mesophyll which surrounds the capsule just within the hypodermis (*fig. 33*).

In the region near the basal pit the inner hypodermis seems to be pretty sharply separated from the cells of the inner portion of the basal wall (*fig. 33*), running down from the dorsal side of the capsule as a very thin layer which abuts against the overlapping edge of the outer layer of the basal wall. The cells of the inner portion of the basal wall in this region are thin-walled and have small intercellular spaces between them, suggesting thus the tissue that was described by Russow as filling the "lens-shaped space" in this part of the capsule of Marsilia. There is, however, no indication of a duplication of the hypodermis to shut off these cells from the rest of the capsule (see Russow '72 or Johnson '98), nor is there any trace of the rod of brown cells described by Russow as occupying the anterior end of this lens cavity.

THE VASCULAR BUNDLE SYSTEM.

This system has been carefully studied by Meunier, and since my own work confirms his in all essential points, I have, with his consent, reproduced several of his figures (*figs. 36–38*) of its mature anatomy.

In the development of the vascular system we have already seen that the axial bundle of the stalk comes from section I entirely, and this is true also of the simple continuation of this bundle into the capsule (*a b*, *fig. 24*). The rapid modification in shape and position of the various parts of the capsule, however, makes it practically impossible to trace out the origin of the many branches in the capsule with reference to segments and meristem layers, as it was possible to do in Marsilia.

After penetrating unbranched nearly to the base of the sori (*fig. 33*), the axial bundle divides, sending one branch to the

right side of the capsule and one to the left, forming a short transverse bundle perpendicular to the main trunk (*tb*, *figs. 36, 37*). Each end of the transverse bundle soon divides again, sending one branch upward and dorsally in each case, and the other downward and ventrally (*figs. 32, 33*). Then each of these four branches, which correspond to what I have called the "lateral branches of the dorsal bundle" in Marsilia, divides to form the three peripheral bundles of its respective valve (*l b f, figs. 30, 36, 37*). Of these bundles, the middle one of each valve gives rise, a short distance above its base, to a short branch (*p a b r, fig. 36*) that turns abruptly into the capsule to join the placental bundle which runs through the length of the placenta just back of the sporangia (*p a b, figs. 30, 36*). Of the other two main branches in each valve, one runs along close to each edge of the latter (*l b f, figs. 27–30*), and all three fuse again at the tip of the valve (*figs. 36, 38*). There is never a fusion of bundles from upper and lower sori on the same side of the capsule as occurs in Marsilia, and the absence of this allows the separation of upper and lower valves on each side, just as the absence of fusion across the median plain in Marsilia allows the separation of the wall of the capsule into right and left valves.

THE SORI.

In the young soral segments the growth in a tangential direction of sections II and V is comparatively slight, while the sections dorsal to the marginal cell grow vigorously and thus push this cell around into a nearly ventral position (*m c*^{1}, *fig. 19*), After increasing considerably in size, the marginal cell divides into halves by an anticline parallel to the median wall (*s p c, fig. 22*), and then these halves are divided further by walls parallel to the first (*figs. 23–26*) and by others perpendicular to these (*figs. 27–29*), giving rise thus to the large number of sporangium mother cells of the sorus. During the growth and division of these derivatives of the marginal cell they are turned over, by the continued growth of the dorsal sections, so that the originally outer surface finally faces toward the cells of section V, which

have been turned in a similar way to face laterally outward (*s p c*, *fig. 23*). The slight depression thus formed on the ventral surface (*s c*, *figs. 23*, *24*) is the beginning of the soral canal, which grows constantly deeper as the capsule develops (*figs. 24–26*), becoming crescent-shaped in cross section (*figs. 27–30*), and finally closing at the outer end, by the growing together of sections V and VI, to completely inclose the sporangial cells (*figs. 26–32*).

By the striking change in the external form of the capsule already mentioned, the position of the sori within this is considerably affected. From their origin (*fig. 14*) we might expect the sori to face the median wall, as in Marsilia, but this is not the case, for in accommodating themselves to the globular form of the capsule, the sori of the upper pair soon come to face inward and downward, while those of the lower pair face upward and inward (*figs. 27–29*), or, in other words, all four face toward the central axis of the capsule.

When fully developed to sporangium mother cells the derivatives of the marginal cells are elongated perpendicularly to the surface of the sorus, densely filled with contents, and have large nuclei in which, in the resting stage, the chromatin is collected in a few rounded masses (*figs. 26–28*). The number of sporangium mother cells in the length of the sorus is ten or more (*fig. 26*), while the number seen in transverse sections varies from four to five at the base or top (*figs. 27*, *30*) to as many as twelve at the middle (*fig. 29*).

The basal cells of the sorus are the first to form sporangia (*fig. 26*), and this begins in each of them by the occurrence of inclined walls, cutting out a tetrahedral apical cell (*s p*, *figs. 26*, *28*, *29*). One or more series of segments are cut off from this, and then a pericline appears at the outer end, completing the sporangium wall and forming the archesporium, which later cuts off the tapetum in the usual way (*s p*, *figs. 28–30*). According to Meunier, who has carefully described the development of the sporangia, the microsporangia and macrosporangia are just alike up to the time of formation of the mother cells. This view is

confirmed by Campbell, and I believe it to be true, though I have not been able to follow out in detail this part of the development.

Among the cells at the base of the sorus, where the first sporangia are formed, are a few cells which do not develop sporangia until much later, so that the sporangia in this region differ greatly in age, and hence in size (*s p*, *fig. 30*). In the middle and upper portions of the sorus there seems to be much less disparity in the size of the sporangia, as all of the sporangial cells form sporangia at about the same time (*fig. 29*). From the similarity in distribution in the sorus of the sporangia first formed to that of the macrosporangia of the mature sorus, one is tempted to believe that these primary sporangia near the base of the sorus are the only ones that develop to macrosporangia, while the backward sporangia, including a few at the base and all those of the upper part of the sorus, give rise to microsporangia only, but this view could not be definitely confirmed.

I can corroborate Campbell's statement that no stalk cell is regularly formed in the development of the sporangia, and whether the cell sometimes seen at the base of the sporangial cell (*fig. 29*) is the homologue of the stalk cell of the Polypodiaceæ seems open to question.

We have seen that while the indusium which separates the sori of the laterally opposite pairs from each other is formed by the ventral outgrowth of sections II and V, that which separates the upper and lower sori of the same side must be derived principally from the sterile marginal cells. The presence of intercellular spaces along the median wall is noticed very early (*i s c*, *figs. 25–27*), and these finally run together to complete the separation of the indusia of the opposite sori. A similar splitting apart begins a little later between the upper and lower sori of the same side.

The fact that these layers of tissue surrounding the individual sori are not originally separate is used by Goebel as evidence for the view that it is not an indusium morphologically, but it seems to me that, just as in Marsilia, the mode of development

by the outgrowth of surface cells favors the opposite view, as has been pointed out by Meunier.

SUMMARY AND CONCLUSIONS.

The leaf of Pilularia develops, like that of Marsilia and many other leptosporangiate ferns, by a two-sided apical cell arising on the right and left sides alternately of the dorsal surface of the stem, near the apex.

The eleven or more pairs of segments formed by this apical cell divide primarily into three sections and a quaternary marginal cell, instead of five and a marginal cell of the sixth grade, as in Marsilia. Each of these four divisions takes part in the formation of all three meristem layers. The sheath of the axial bundle is derived from the procambium and not from the ground meristem as in other ferns. The mesophyll of the mature leaf is of a single layer. Outside of this are the ten air canals, separated both laterally and transversely by perforated partitions, and surrounded externally by the epidermis and hypodermis developed from the protoderm.

No indication of a rudimentary lamina could be found by carefully following the details of division in the terminal segments of the circinately coiled leaf.

The sporocarp of Pilularia is a branch of the leaf, arising in an anterior marginal cell at the base of the latter. It grows by a two-sided apical cell which cuts off six or more pairs of segments, and is then divided up by irregular anticlines. These segments, like those of the sporocarp of Marsilia, form seven primary divisions.

This plan of division found in both genera must, I think, be regarded as a characteristic of the spore-bearing portion of the leaf in their common ancestor. From the absence of a lamina in the sterile leaf of Pilularia, it might be suggested that the sporocarp, which also possesses no lamina, had been derived from a leaf of this type. For phylogenetic reasons, however, we must believe that the leaf of the Leptosporangiatæ, from which the Marsiliaceæ are derived, possessed á lamina, developed

in the same manner as in many other families of the group, and had a petiole more like these same forms in the number of divisions of its segments (see Sadebeck '74). Pilularia, on the contrary, has no lamina, and has a smaller number of divisions in the leaf segments, resembling in this respect certain petioles of Marsilia (Johnson '98), where the number of sections is reduced below the normal. Again, the type of division in the capsule seems to be easily derivable from that of the leaf of Marsilia, for the interpolated section IV of the former seems so plainly to assist in pushing the marginal cell around to the ventral surface, that we can readily believe it to have been added to a leaf of this type for this particular purpose. When all of these facts are considered, it seems evident that, so far as the structure of the leaf indicates, Marsilia is the less modified of the two genera, and resembles the other leptosporangiate ferns more closely, while Pilularia has a leaf very much reduced from the ancestral type. Whether the capsule of Pilularia is derived from one with more numerous sori cannot, perhaps, be profitably discussed until we know the details of development in such forms as *Marsilia polycarpa*, or *M. Aegyptiaca*, and *Pilularia minuta*, where the number of sori is reduced in each genus below that in the forms already studied, but from my own study of *P. globulifera* and *M. quadrifolia*, I am inclined to think the capsule of the latter, like its leaf, is the more primitive of the two. It seems to me that detailed study of the leaf development throughout the Leptosporangiatæ may be expected to give more light on the exact affinities of the Marsiliaceæ with the other families of the group.

In the segments forming the stalk of the sporocarp the protoderm gives rise to epidermis and hypodermis, and the ground meristem to mesophyll and the irregular partitions separating the small air canals, while the procambium gives rise in section I to the vascular bundle and in the other sections to the ventrally placed stereome bundle.

The remaining younger segments of the sporocarp are devoted to the formation of the capsule. In this region two of the ulti-

mate marginal cells on each side are devoted to the formation of sporangia, all of those of each sorus arising from one of these four sporangial marginal cells. The sori thus arise in right and left pairs, one above the other on each side, and are not terminal in origin, as described by Meunier, nor with two sori on the median plane, as indicated by Campbell. These sporangial marginal cells give rise in a way somewhat similar to that found in Marsilia, to the large number of sporangium mother cells of the sori, and are in the meantime surrounded by the more vigorous growth of the other portions of the ventral side of the capsule. By the more rapid growth at the base of the capsule on the ventral side, the openings of the soral canals thus formed are pushed around from a lateral position to become nearly terminal in the mature sporocarp.

The macrosporangia and microsporangia are not derived from different marginal cells, as in Marsilia. The earliest evidence of differentiation found here is in the fact that the first sporangia formed, most of them near the base of the sorus, seem to develop macrosporangia, while the upper and younger ones become microsporangia. Except in this matter of location, the two kinds of sporangia are just alike up to the formation of the spore mother cells.

The outgrowth of the cells of the ventral surface of the capsule gives rise not only to the wall surrounding all of the sori, but also to the so-called indusium which separates the different sori from each other. It seems to me that Meunier is right in saying that the development of this tissue here (as in Marsilia also) is sufficient warrant for calling it an indusium. But we cannot agree with Campbell in regarding these indusia as the inturned edges of leaflets enclosing the sori, since we have no evidence that any structure homologous with the lamina occurs in the capsule.

Of course, the whole question of homology is complicated by the fact that the sporangia of the Marsiliaceæ seem to arise on the ventral surface of the leaf. But if we pass from forms like Asplenium, with sporangia borne near the middle of the

dorsal surface of the leaf, through forms like Adiantum, with sporangia near the edge on the same surface, to Lygodium, where, according to Prantl ('87), the sporangia actually arise from marginal cells, the transition to the Marsiliaceæ, with several sporangia arising from each marginal cell, does not seem to be so very abrupt. The indusium of Lygodium also seems to have a striking resemblance to that of the Marsiliaceæ in some features of its development, and may repay further investigation from this point of view.

The axial vascular bundle entering the base of the capsule divides into two, one branch going to the right and the other to the left side of the latter. Each of these again divides, forming four branches, each of which furnishes the three main bundles of a sorus. The middle one of the three in each case develops a placental branch which connects with the placental bundle present in the axis of the placenta. The three bundles of each sorus fuse together at the tip of the valve, but there is no fusion of the bundles of the upper and lower sori on the same side like that found in Marsilia.

The firm wall of the globular capsule of Pilularia is made up, like that of Marsilia, of an epidermis of thick brown-walled cells with trichomes and stomata scattered among them. Within this are two hypodermal layers, the outer of very thick-walled, regularly prismatic cells, and an inner layer of larger, more irregular, brown-walled cells. Across the base of the capsule is formed the thick basal wall, the outer layer of which is continuous with and exactly like the outer hypodermis and has the same "light line" running through it. Near the dorsal side of the capsule there is a narrow slit through this wall, corresponding to the air passage which opens into the lens-shaped space in Marsilia, but, though a tissue similar to that found in this space is present in Pilularia, it is not cut off from the rest of the capsule by a duplication of the hypodermis.

Just opposite the basal wall there is a depression in the dorsal surface of the sporocarp, and just below this, at the upper end of the stalk, is an outgrowth corresponding to the lower

tooth of the capsule of Marsilia. The upper tooth, which in Marsilia consists simply of elongated epidermal cells, is entirely wanting in Pilularia.

In conclusion, the sporocarp of *Pilularia globulifera* is essentially the equivalent of a Marsilia sporocarp in which the number of sori has been reduced to two pairs, and will probably be found to correspond even more closely in development with those Marsilias, like *M. polycarpa* or *M. Ægyptiaca*, which also have a small number of sori. Morphologically, then, we must in both cases consider the capsule as equivalent to a branch of the leaf in which the marginal cells have been devoted to the formation of sporangia instead of a lamina.

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EXPLANATION OF PLATES I–III.

Abbreviations used.—*A*, apex; *ab*, axial bundle; *ac*, air-canal; *arc*, archesporium; *bp*, basal pit; *bs*, bundle sheath; *bw*, basal wall; *cp*, transverse partition; *D*, dorsal; *d*, protoderm; *dw*, protoderm-wall; *ep*, epidermis; *F*, sporocarp; *ha*, halving anticline; *hy*, hypodermis; hy^1, outer hypodermis; hy^2, inner hypodermis; *id*, indusium; *isc*, intersoral cavity; *L*, leaf; *lb*, lateral branch of vascular bundle; *lbf*, fork of the lateral branch; *ll*, light line; *lp*, longitudinal partition; *lt*, lower tooth; *mc*, marginal cell; mc^1, mc^2, etc., marginal cell of the first, second, etc., grade; *mp*, mesophyll; *mw*, median wall; *pa*, placenta; *pab*, placental bundle; *pabr*, placental branch; *pb*, ground-meristem; *pc*, partition cell; *pl*, procambium; *plw*, procambium-wall; *pp*, pores in partition; *S*, stem; *sc*, soral cavity; *scl*, stereome bundle; *sp*, sporangium; *spc*, sporangial cells; *st*, stoma; *sw*, segment wall; ta^1, first transverse anticline; *tc*, trichome; *tp*, tapetum; *tr*, trachea; X, apical cell; I, II, etc., first, second, etc., section walls.

All figures are camera drawings from microtome sections, except *figures 36–38*, which are copied from Meunier.

PLATE I.

FIG. 1. Transverse section of stem through apical cells of two young leaves. × 400.

FIG. 2. Ventral surface of tip of young leaf. × 400.

FIG. 3. Lateral surface of same. × 400.

FIG. 4. Part of transverse section of young leaf. × 400.

FIG, 5. Similar section of an older leaf. × 400.

FIG. 6. The same still older. × 400.

FIG. 7. The same older than the last. × 400.

FIG. 8. The same still older than the last. × 400.

FIG. 9. Similar section of nearly mature leaf. × 75.

FIG. 10. Part of tangential section of a young leaf through the partitions and air-canals. × 200.

FIG. 11. Similar section of older leaf. × 200.

FIG. 12. Part of transverse section of stem showing ventral surface of a young leaf and the mother cell of a sporocarp. × 400.

FIG. 13. Dorsal surface of tip of a young sporocarp. × 400.

FIG. 14. Ventral and lateral surface of the same. × 400.

FIG. 15. Surface view of the apex of an older sporocarp, showing the fate of the apical cell. × 400.

FIG. 16. Part of a section of the tip of a young sporocarp, which cuts the segment on the right parallel to the upper and lower segment-walls and the segment on the left perpendicular to these. × 400.

FIG. 17. Part of a transverse section of a young sporocarp. × 400.

FIG. 18. Similar section of slightly older sporocarp. × 400.

FIG. 19. Part of transverse section of a capsule showing ultimate marginal cell. × 400.

PLATE II.

FIG. 20. Transverse section of stalk of sporocarp. × 400.

FIG. 21. Similar section of an older stalk. × 300.

FIG. 22. Transverse section of a capsule older than that shown in figure 19. × 400.

FIG. 23. Similar section slightly older than the last. × 400.

FIG. 24. The same still older, though lower sori. × 400.

FIG. 25. Approximately horizontal section through upper pair of sori of a capsule considerably older than that shown in figure 31. × 200.

FIG. 26. Part of similar section slightly older, showing formation of sporangia. × 400.

FIG. 27. Part of a section transverse to the sori of a capsule of the age shown in figure 31, near the ventral surface. × 400.

FIG. 28. Similar section of right upper sorus a little older. × 400.

FIG. 29. Similar section near the middle of left upper sorus still older. × 175.

FIG. 30. Similar section, near the base of lower right hand sorus, older than last. × 175.

PLATE III.

FIG. 31. Approximately sagittal section of a young sporocarp, just at the right of the median plane. × 400.

FIG. 32. A similar section of a much older sporocarp. × 200.

FIG. 33. Nearly median sagittal section of a mature sporocarp. × 30. (At *lbf*, in dotted lines, the vascular bundles which would be seen in a section a little farther from the median plane than this.)

FIG. 34. Horizontal section through pit and base of mature capsule, just dorsal to slit in basal wall. × 175.

FIG. 35. Section parallel to last through slit in basal wall. × 175.

FIG. 36. View from the ventral side of the vascular bundle system of the mature capsule. × 15. (The arrow indicates the direction of the median plane.)

FIG. 37. View from above of the transverse bundle at the base of the capsule and its branches. × 15.

FIG. 38. View from above of the bundles at the top of the capsule. × 15.